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LOW TEMPERATURE AND MOISTURE AS FACTORS IN THE ECOLOGY OF THE RICE WEEVIL *SITOPHILUS ORYZA* L. AND THE GRANARY WEEVIL *SITOPHILUS GRANARIUS* L.

BY

WILLIAM ROBINSON

L. C.

A THESIS

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

JUNE 1926

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LOW TEMPERATURE AND MOISTURE AS FACTORS IN THE ECOLOGY OF THE RICE WEEVIL, *SITOPHILUS ORYZA* L., AND THE GRANARY WEEVIL, *SITOPHILUS GRANARIUS* L.

By William Robinson

INTRODUCTION

The factors of temperature and moisture occupy a vitally important place in the environment of any organism. In their effects on the individual these factors are so closely inter-related that a complete study of one can not be made without regard to the other. This treatise presents a study of the comparative susceptibility of two species of grain weevils to low temperatures and to varying amounts of moisture. Each factor is treated separately by holding one constant and varying the other.

The investigation has been facilitated by the excellent equipment of the Division of Entomology and Economic Zoology for studying the effects of temperature and moisture.

The type of insects used in this research has greatly lightened the routine work. Grain weevils appear to possess some advantages in the study of certain phases of biological phenomena, not the least being the ease with which they may be obtained at all times of the year. They require very little attention, and with a slight amount of care may be bred in the laboratory by thousands. They are also easily handled. Their food requirements are simple, and when once satisfied—with a "measure of wheat"—they will maintain and reproduce themselves throughout the year in countless numbers. With experiments, as in the series reported on, in which quantity determinations add special value to the data, grain weevils (within the limits of their adaptability) have much to recommend them.

Acknowledgments are due to Dr. R. N. Chapman, chief of the division, at whose suggestion the study of the comparative resistance of the grain weevils to low temperatures was begun and who conceived the idea of controlling by low temperatures weevils infesting stored grain; to Dr. C. H. Bailey, of the Division of Agricultural Biochemistry, and to Dr. Anthony Zeleny, of the Department of Physics, for their kind assistance in certain phases of the problem.

TEMPERATURE

GRAIN WEEVILS AND THEIR NORMAL RELATIONS TO LOW TEMPERATURES

Throughout their entire life cycle, grain weevils and rice weevils live normally in stored grain. In this environment they are not exposed to extremes of low temperature, for grain in storage is a poor conductor of heat. It absorbs and gives off heat very slowly and can remain all winter above the freezing point of water. Thus the matter of encountering and surviving the cold of winter—a problem of the first importance to many insects—is one to which they have no need to adapt themselves. Having developed no resistance to cold, they afford an opportunity of studying the effects of low temperatures upon an organism which can do nothing to offset its injuries.

PREVIOUS WORK ON RESISTANCE OF GRAIN WEEVILS TO LOW TEMPERATURES

Dendy and Elkington (1920) exposed both species to temperatures of 33°-36° F. for 11 days and found that 9 per cent of *granarius* and 97 per cent of *oryza* were killed.

Back and Cotton (1924) conducted an extended series of experiments upon the two species, exposing them to a graded series of temperatures ranging from 0° F. to 60° F. They found *granarius* in every instance to be the more resistant. From their data may be seen also an interesting correlation between the degree of temperature and the time required to kill. That is, by raising the temperature the length of exposure was greatly increased and a line representing this correlation would take the form of a hyperbola.

STOCK CULTURE OF WEEVILS

In the present series of experiments it was endeavored to rear the insects under as nearly uniform conditions of temperature and moisture as was possible in the laboratory. Metal tanks 30 inches high and 30 inches in diameter, each containing several bushels of wheat, were used. In summer the temperature of the laboratory ranged from 70° to 80° F. and in winter between 65° and 75° F. In summer, the moisture content of the wheat in the tanks remained from 13 to 14 per cent; but in winter, with a relative humidity as low as from 25 to 10 per cent, wheat exposed in the laboratory dried down to 10 per cent moisture. However, a pan of water in each tank prevented the moisture content of the wheat from going below 13 per cent. Weevils were not allowed to accumulate in the tanks in large numbers, as that tended to change both the moisture content and the temperature of the wheat.

METHOD OF OBTAINING WEEVILS FROM THE STOCK CULTURE

The means of obtaining the insects in large numbers from the mass of wheat in which they live was very simple and effective. When grain weevils are disturbed they come to the surface of the grain and climb the sides of the container, so the grain was stirred thoroly and when a large number of individuals had collected on the sides they were swept with a brush on to a piece of cardboard shaped to fit the inside of the tank. The granary weevil has more difficulty than the rice weevil in climbing a smooth surface, so the inside of the granary weevil tank was lined with rough paper.

TECHNIC OF HANDLING WEEVILS THROUGHOUT TESTS

After the weevils had been collected they were transferred to glass vials which had been cooled to the temperature of the exposure, and then placed in the refrigerating cabinet. The most satisfactory containers were 3 inches high and 1⅛ inches in diameter. Each was fitted with a cork stopper having a ⅜-inch hole covered with wire screening. They were easily and quickly handled and occupied little space.

A record of each experiment was made on a form similar to that shown in Figure 1. As each container was removed from the refrigerator, the weevils were placed in a petrie dish 5 inches in diameter and ½ inch high and were counted while still dormant. By the use of petrie dishes the activities of the insects could be observed without disturbing them. This was necessary in taking counts because of their habit of feigning death when disturbed. The effect of exposure was classified as follows: (1) motionless, (2) feebly kicking, (3) feebly crawling, (4) actively climbing. These divisions were arbitrary and not always well marked, as there were frequent gradations from one to the other, but in general they were satisfactory. They also provided a means of recording quickly the effect of insufficient exposure, for, as mentioned later, it was planned to run a graded series of exposures at each given temperature in order to study the effect of a partial exposure.

Observations on the condition of the weevils in the petrie dishes were made until they became motionless or regained activity; and no experiment was closed until columns 2 and 3 in the chart could be marked with a zero. This sometimes required from two to five days. A few grains of wheat were added to maintain the active weevils. When each experiment was closed the insects were destroyed, and none were used for more than one experiment, as it was thought possible that the previous treatment might affect their resistance to subsequent exposures.

Checks.—Check experiments were conducted for every series of exposures. As soon as a series was placed in the refrigerator, at least 3 checks, each containing about 150 individuals, were prepared from the same stock culture and kept under normal conditions of temperature and moisture. When the series of exposures was terminated, the dead in the checks were counted and all were destroyed. No individuals in the checks were used a second time. In all, 47 check experiments, or 7582 individuals, were used. The mortality was always very low, even in exposures of 30 or more days, and never exceeded 2 per cent.

EFFECT OF LOW TEMPERATURES ON GRAIN WEEVILS
Series A-1

Experiment No... 394 ..... Container No. 19 .... Species: granarius * oryza

Exposure Started: Day.... Oct. 29 ..... Hour..... 5:30 p.m. .....

Exposure Finished: Day.... Oct. 31 ..... Hour..... 1:30 p.m. .....

Length of Exposure: Days..... 1 ..... Hours..... 20 .....

Temperature to be dropped to ..... 20 °F Cabinet..... C. .....

Any Variation in Temperature?.... No ..... Mean Temperature..... 20 °F

Individuals in Experiment... 231 ...No. Killed... 162 ... % Killed.. 70.1 .....

Individuals in Checks..... 148-152-162 ..No. dead in Checks at end of Exp. 2-2-1 ....

Activities after Exposure

TIME in hours after exposure	Motionless	Feebly Kicking	Feebly Crawling	Actively Climbing
24	221	10	-	-
72	136	others	6	-
96	92	"	8	3
120	81	"	3	17
160	others	-	-	69

Remarks:

Fig. 1. Form Used in Recording Details of Exposures

Refrigerating outfit.—Low temperatures were obtained by a two-ton automatic refrigerating machine and a constant temperature and humidity cabinet with a recording wet and dry bulb thermometer. This equipment gave the utmost satisfaction, and temperatures varying not more than one-half of a degree Centigrade were obtained regularly, even over extended periods.

Constant temperatures.—The uniformity of the temperatures, as mentioned above, which were obtained during the experiments, was of special value, as no data were available on the effect of constant temperatures upon these two species of insects. In the work of Back and Cotton (1924) temperatures approaching constancy were used, but even there a spread of at least 5° F. usually occurred. Later it will be shown that a difference of 5° F. in the temperature materially affects the percentage of mortality at any given length of exposure.

Number of weevils used.—It was discovered early in the work that, despite the fact that the weevils were obtained from a common culture, the resistance of individuals to low temperatures varied considerably. Large numbers, consequently, had to be used in order to present as accurate data as possible. The rapidity with which both species, especially *oryza,* increase always assured a plentiful supply for the experiments. In the series of experiments on low temperatures, 129,477 insects were used; and in those on moisture, over 60,000 were used.

Full and partial exposures.—The time necessary to obtain 100 per cent mortality was determined for some experiments, while for others it was desired to note the effect of shorter exposures upon the percentage of mortality. Thus, removals from the refrigerator were begun early and were continued at intervals until full exposure had been made.

Abrupt and gradual exposures.—In one series of experiments, weevils were transferred directly from the stock culture to the low temperature at which they were to be tested. In other series they were brought down gradually by decreasing slowly the temperatures between dormancy and the final exposure. The latter procedure was planned to determine if the process of "hardening" observed by Payne (1926) with oak borers would increase the resistance of these grain weevils to low temperatures.

Range of temperatures used in abrupt exposures.—Beginning with the temperature at which each species became dormant, the following range of temperature was used:

TABLE I

RANGE OF TEMPERATURE USED

S. oryza		*S. granarius*	
Degrees C.	Degrees F.	Degrees C.	Degrees F.
7.2	45	1.6	35
1.6	35	−1.1	30
−1.1	30	−6.6	20
−6.6	20	−12.2	10
−12.2	10	−17.7	0
−17.7	0		

For each of these temperatures about 25 experiments were run, each having between 150 and 250 individuals.

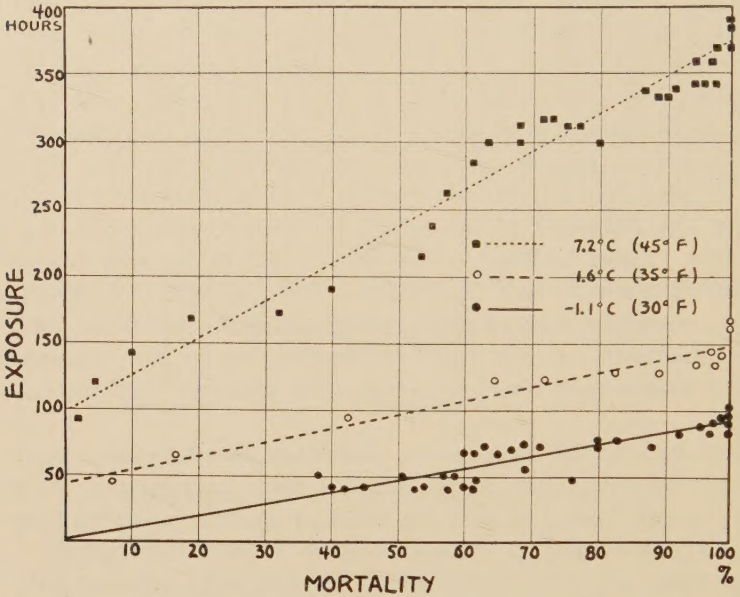

Fig. 2. Percentage of Mortality Obtained with *S. oryza* at 7.2°, 1.6°, and —1.1° C.

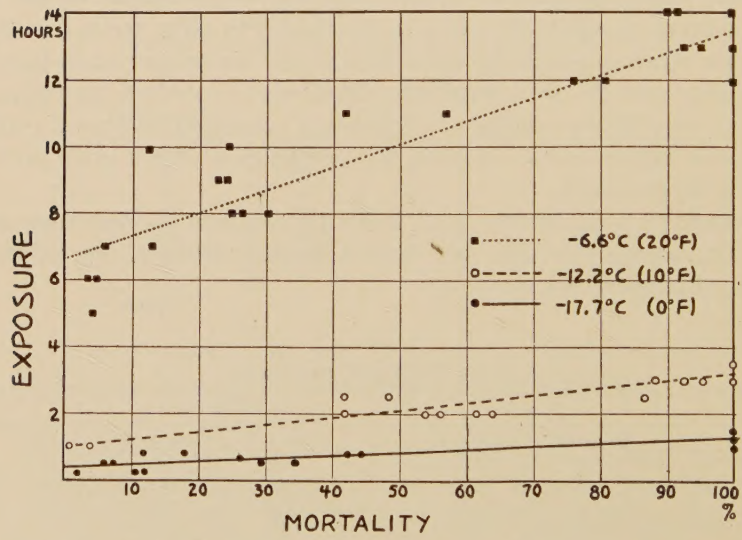

Fig. 3. Percentage of Mortality Obtained with *S. oryza* at —6.6°, —12.2°, and —17.7° C.

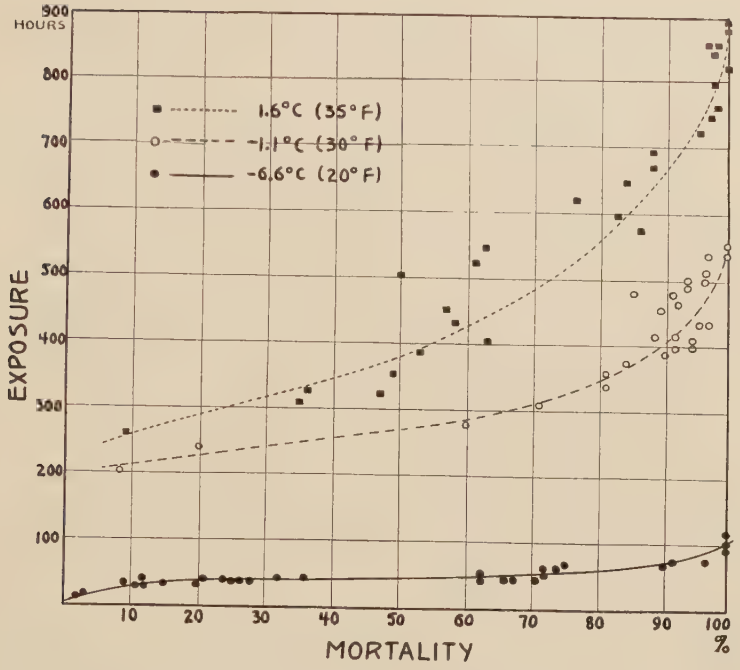

Fig. 4. Percentage of Mortality Obtained with *S. granarius* at 1.6°, —1.1°, and —6.6° C.

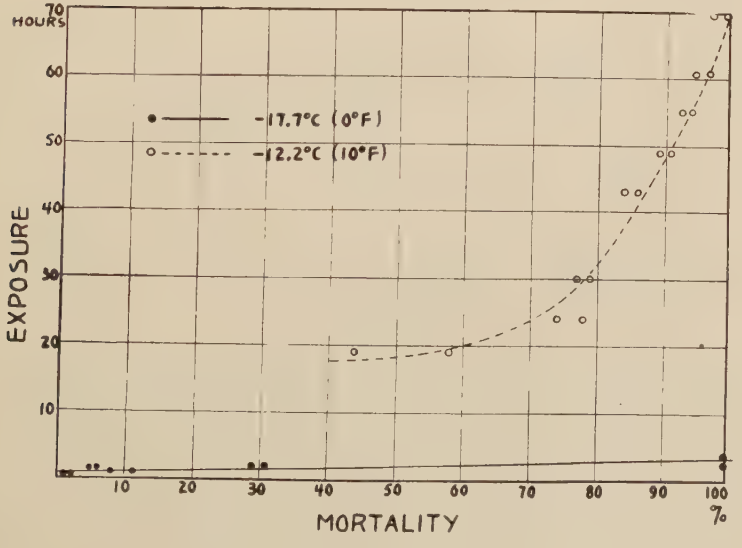

Fig. 5. Percentage of Mortality Obtained with *S. granarius* at —12.2° and —17.7° C.

Discussion of results of abrupt exposures.—The effects on *oryza* of the graded series of low temperatures are shown in Figures 2 and 3; and of those on *granarius*, in Figures 4 and 5. These charts show the duration of exposures in hours. The time required to obtain 100 per cent mortality is a great deal less than was found necessary by Back and Cotton (1924). A possible explanation of this difference is that the moisture content of their stock cultures might have been considerably higher than that used in these experiments. Here the weevils were reared in cultures of wheat having approximately 13 to 14 per cent moisture content. It is shown later, in the discussion of moisture as a factor, that the moisture content of the wheat has a marked effect upon the resistance of these insects to low temperatures.

A well marked correlation between length of exposure and percentage of mortality for any given temperature, is observable in all the charts. The curve representing this correlation is practically a straight line for *oryza* and a curved one for *granarius*. It is evident that an increase or a decrease in the length of exposure, within the limits of the experiment, would bring about a corresponding change in the percentage of mortality.

Exposures required to obtain full mortality at the different temperatures used are given in Tables II and III. These also show that the lower the temperature the shorter is the necessary exposure. A correlation curve for time and temperature would probably take the form of a hyperbola, as observed for Back and Cotton (1924).

TABLE II

TIME REQUIRED AT VARIOUS TEMPERATURES TO KILL *S. oryza*

Fig. No.	Temperature		Av. length of exposure, hrs.	No. of experiments conducted	No. of individuals used
2	7.2° C.*	(45° F.)	350	39	10,534
2	1.6	35	160	15	2,306
2	—1.1	30	98	72	16,278
3	—6.6	20	14	58	15,374
3	—12.2	10	3½	24	3,243
3	—17.7	0	1½	32	3,519

* Temperature at which dormancy occurs.

TABLE III

TIME REQUIRED AT VARIOUS TEMPERATURES TO KILL *S. granarius*

Fig. No.	Temperature		Av. length of exposure, hrs.	No. of experiments conducted	No. of individuals used
4	1.6° C.*	(35° F.)	875	32	7250
4	—1.1	30	545	36	5359
4	—6.6	20	100	42	7170
5	—12.2	10	70	22	3026
5	—17.7	0	2½	28	4291

* Temperature at which dormancy occurs.

The greater susceptibility of *oryza* to low temperatures, noted by previous workers, is confirmed. This difference in resistance of the two species to cold probably accounts in part for the fact, as mentioned by Cotton (1921), that *oryza* is found more abundantly in the South;[1] while *granarius,* altho a pest of stored grain in warm climates, can also be found well distributed northward.

Inability to endure dormancy.—These insects came originally from the tropics, it is believed, where, not having to endure the cold of winter as in the temperate and frigid zones, they apparently never developed the ability to hibernate; or, having once possessed this characteristic, have lost it through disuse. Living normally in stored grain, the temperature of which fluctuates very little and seldom goes below the freezing point of water even during our northern winters, it has been possible for these two species to spread north and south and still to be protected largely from low temperatures.

If the temperatures at which these insects normally live be gradually diminished, a corresponding decrease in the rate of their activities will occur until at approximately 7.2° C. (45° F.) with *oryza* and 1.6° C. (35° F.) with *granarius,* the weevils collapse and become motionless. They make no effort to avoid the approach of low temperatures, neither are they stimulated into assuming any resistant condition; they merely move more and more sluggishly as the environment becomes colder and finally fall into a state of dormancy. Neither species can endure this condition. *Oryza* perishes in 400 hours, approximately 17 days; and *granarius* in 930 hours, approximately 38 days.

Effect of low temperatures upon weight.—During the course of exposure to various low temperatures, the weight of 500 weevils of each species was taken daily until deaths began to occur. From the first day a loss of weight was noticed, and this decrease was so nearly constant that the daily loss could be closely predicted. The correlation between percentage of weight and length of exposure is shown in Figure 6. This is a straight line correlation for both species at the temperatures at which they become dormant. With the approach of death a slight increase in loss occurred. At these temperatures *granarius* lost 18 per cent and *oryza* 24 per cent of their original weight. This coincides with the fact that *granarius* can endure dormancy for a longer period.

A comparison is also made of the loss of weight of *oryza* at two different temperatures. At 7.2° C. (45° F.) the loss is 24 per cent at time of death, while at 1.6° C. (35° F.) it is only 12 per cent. This indicates that other factors besides the depletion of the body tissues by metabolic processes are responsible for the inability of these insects to endure dormancy.

[1] In the observations on moisture, it is mentioned that the difference in susceptibility to low moisture content of wheat may affect in part the northerly distribution of the two species.

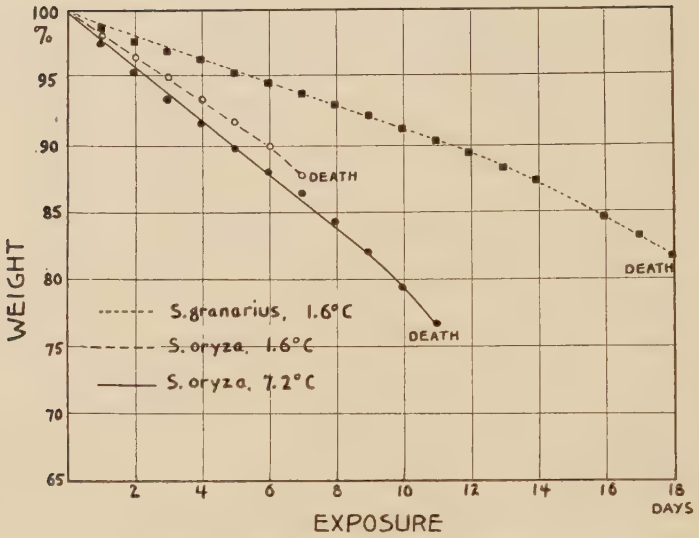

Fig. 6. Loss of Weight During Dormancy

Tests were made, for a check, on the loss of weight of each species when kept without food at room temperature, approximately 22.2° C. (72° F.). The weevils were all unusually active and seemed to be made very restless by the absence of food. Their activity continued until exhaustion set in. Death began to occur on the sixth day, at which time this experiment was terminated. As with the other tests, the straight line correlation is evident (Fig. 7). The loss of weight with *oryza* was 44 per cent and with *granarius* 33 per cent.

This marked and continuous loss of weight doubtless explains in part why these insects are unable to endure dormancy. Their inability can be attributed to a persistence in the rate of metabolism even while exposed to low temperatures. The reason for this is unknown. Rasmussen (1916) reviews a number of theories advanced by various workers to account for the phenomenon of hibernation during which period metabolism and the consequent breaking down of tissues are reduced to an extremely low rate. He states, however, that all the theories are based upon insufficient data, hence many of them are of little value. The same author (1923) reviews the extensive literature on the so-called hibernating gland of mammals which has been claimed by some writers to secrete a substance of significance to hibernating animals. Here, also, this author states that the evidence as to this gland having any bearing on hibernation is inconclusive. Thus, in the absence of precise data on the cause of dormancy in hibernating animals, it is especially difficult to determine what is lacking in animals which are killed by low temperatures because they can not hibernate.

Discussion of the gradual exposures.—To determine if grain weevils could withstand low temperatures for a longer period if exposed to them gradually, the following tests were made. From the stock culture, which had a temperature of 21° C. (70° F.), 11,162 rice weevils were placed in standard containers with wheat from their culture. They were then exposed in the refrigerator to 10° C. (50° F.) for 72 hours, during which time they moved very sluggishly in the wheat. Further reductions were made—to 7.2° C. (45° F.) for 65 hours, to 4.4° C. (40° F.) for 48 hours, and to 1.6° C. (35° F.) for 36 hours. The time in each case was calculated to be not more than one-sixth the fatal exposure, and it was expected that none would perish. This was preparatory to a final exposure of —1.1° C. (30° F.), at which temperature the test of resistance was to be made.

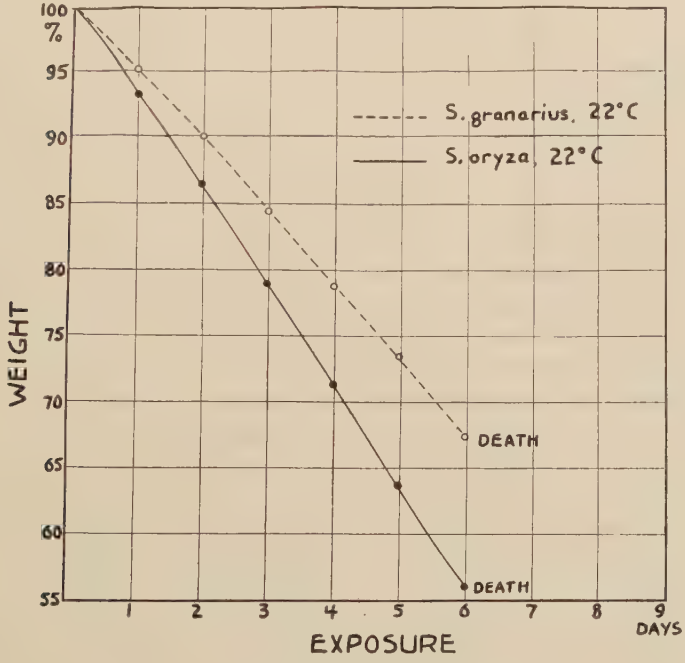

Fig. 7. Loss of Weight During Activity at Room Temperature, 22° C., No Food Being Supplied

Each time the temperature was reduced a container with about 200 individuals was removed permanently from the cabinet. In every case, none of the insects showed any signs of injury and all became active in a few minutes after removal. This is an important point, as it showed that all the insects were alive at the beginning of the final exposure.

When the temperature of —1.1° C. was begun, 32 containers with 8941 rice weevils were placed in the cabinet to serve as checks. These weevils had just been taken from the same culture as the others and had not been previously subjected to low temperatures. The exposure to —1.1° C., therefore, began simultaneously with both series, and from that time on both were treated alike. Containers were removed at short intervals and the results are shown in Figure 8.

It is evident from a study of this chart that instead of their resistance being increased by a gradual exposure to low temperature, these weevils were actually killed in a much shorter time than by an abrupt exposure. For instance, 65 per cent were killed in 36 hours under a gradual exposure, while to obtain an equal mortality with a sudden drop required 95 hours; and to obtain 37 per cent mortality required 24 hours with the former treatment and 72 hours with the latter.

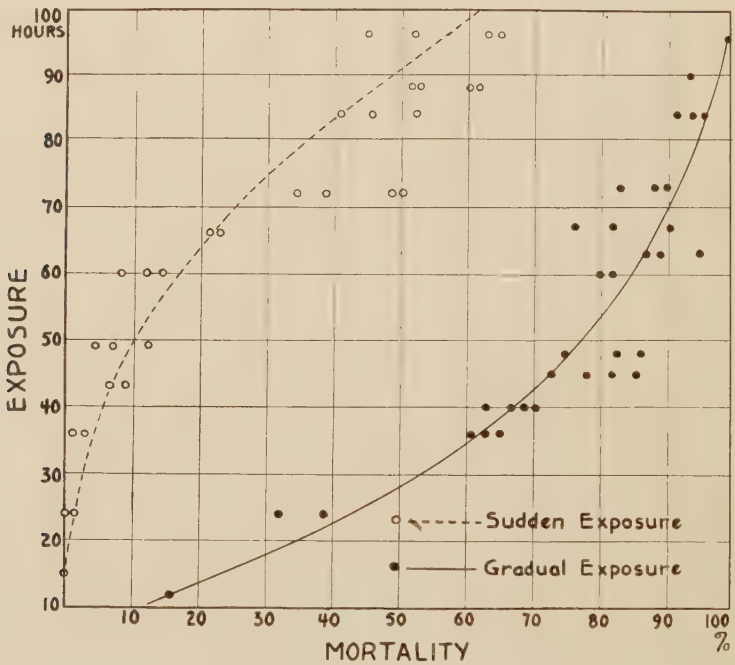

Fig. 8. Comparison of Results Obtained with *S. oryza* Under Conditions of Sudden and Gradual Exposures

The time of the gradual exposure in these experiments was set arbitrarily at about one-sixth of the fatal exposure, and as no mortality occurred during that time the duration of this exposure might have been increased. It seems reasonable to assume that the decreased resistance of the weevils would be proportionate to the length of time

occupied in bringing them down to the low temperatures. This is supported by the fact, as shown in Figure 6, that loss of weight and consequently resistance occurs each day of dormancy.

Thus, the gradual approach of low temperatures which increases the resistance of hibernating insects and enables them to withstand the very low temperatures of winter, decreases the resistance of grain weevils and probably of the entire group of stored products insects.

The wheat in the culture from which these weevils were obtained had 16.4 per cent moisture. This gave the insects an increased resistance, as shown in the discussion of moisture, for it is noticeable that even with the decrease of resistance incurred by the gradual exposure, the percentage of mortality is much the same as that for weevils from a 13.2 per cent culture with a sudden decrease. This reveals the complexity of the study of resistance to low temperatures when the factor of moisture is considered, as it must be.

Cumulative injury and recovery.—In the discussion of dormancy and of the loss of weight during exposure to low temperatures, it was stated that as long as the grain weevils are dormant a continuous injury goes on which finally results in death. To determine if this cumulative injury is of a permanent nature or if it can be checked and overcome before death occurs, the following experiments were conducted. Weevils to the number of 15,614 were placed in the refrigerating cabinet at a temperature of —1.1° C. (30° F.) and exposed for 24 hours. During that period all suffered a loss of weight and an amount of injury was caused which resulted in a 32 per cent mortality.

With this definite injury against them, 4452 were taken out and placed in a culture of wheat having a moisture content of 19.7 per cent and standing at 22° C. (72° F.), room temperature. There, activity was shortly resumed. The remaining 11,162 were left in the refrigerator to determine how long it would require the accumulating injury to bring about death. This was found to be 96 hours. Those which had been transferred to the warm, moist wheat were left there for one week and then returned to the refrigerating cabinet. They had previously been exposed for 24 hours, or exactly one-quarter of the time required to kill them all, and had received injury to the extent of 32 per cent of the total mortality. Therefore it was possible, if the injury were permanent, that in an additional 72 hours all would be dead. It was found in this second exposure, however (Fig. 9), that they exceeded that period and went beyond the total span of the original expectancy, which was 96 hours. When the experiment ceased, 9 per cent were still active at 170 hours.

Thus it is seen that the injury received during dormancy, altho cumulative and eventually resulting in death, can be overcome by a

return to normal conditions. In addition, the original vigor of the weevils can even be increased if the new environment be a more favorable one.

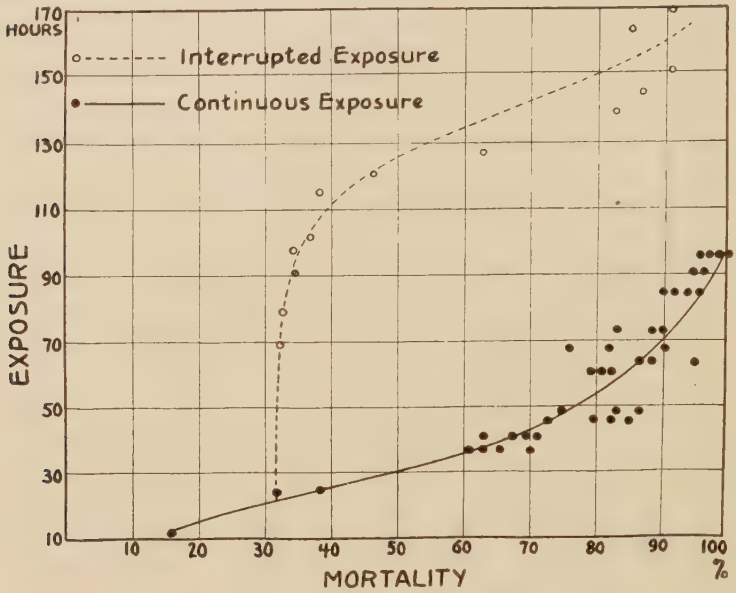

Fig. 9. Results of Interrupted and Continuous Exposures on *S. oryza*

MOISTURE

GRAIN WEEVILS AND THEIR MOISTURE PROBLEM

Moisture, which is necessary to the life of all organisms, is obtained in three ways: (1) directly, as in drinking; (2) through the food; (3) through metabolic changes within the organism. Such insects as stored products pests, which never have access to water directly, have to depend upon obtaining from the last two sources the amount of water needed.

The moisture content of grain varies somewhat, depending partly upon the variety and the degree of curing, also upon the amount of exposure to the air. Grain is affected by the amount of moisture in the air, giving off or taking on moisture until it comes into equilibrium with the surrounding atmosphere. The moisture content of stored wheat is probably between 10 and 18 per cent. This is a limited source of water when compared with the supply available to such insects as grasshoppers and aphids, which feed upon succulent plants containing from 60 to 90 per cent of water. With the former the problem appears to be the conservation, and with the latter the elimination, of

water. Thus, insects which live in stored grain, having access to very limited amounts of water and having adapted themselves to this condition, are affected by slight fluctuations in the moisture content of the grain.

PREVIOUS WORK ON THE RELATION OF GRAIN WEEVILS TO MOISTURE

Cole (1906) states that moisture in the atmosphere and in the food is favorable to both species of weevils and the lack of it is fatal. He does not, however, give precise data. One observation that "the rice weevil (*Calandra oryzae*) is distinctly less sensitive to desiccation than the corn weevil (*C. granaria*) and the former will live twice as long under the same conditions of desiccation as the latter" is not in accordance with the results of experiments conducted here.

Dendy (1918) studied the attractiveness of water in various containers placed in wheat, and concluded that both species are much attracted to moisture.

Dendy and Elkington (1920) found that when exposed to moist air wheat is more subject to attack by weevils than when kept dry. They also state that "very dry wheat is less liable to attack by weevils than wheat with a moderate or high moisture content." In addition, they showed that both species died when fed upon wheat having a moisture content of approximately 9 per cent.

EFFECT OF MOISTURE CONTENT OF WHEAT UPON MOISTURE CONTENT OF GRAIN WEEVILS

One of the earliest tests made here was to determine if the moisture content of the wheat in which the weevils live has any effect upon the moisture content of the body tissues of the weevils. Obviously, the amount of water in the wheat when eaten affects the moisture content of the weevils directly; and the amount of moisture in the grain affects the relative humidity of the air within the bin or container. This in turn influences the rate of elimination of moisture from the bodies of the weevils. It was therefore expected that moist grain would increase the moisture content of the weevils and that wheat containing less than the usual percentage of moisture would cause a corresponding decrease. However, as the experiments show, the opposite conditions were found to exist.

Ten samples of wheat containing (as shown in Tables IV and V) from 20.3 to 4.8 per cent moisture were prepared, and in these were placed a large number of weevils of each species. Readings of the moisture content of the weevils were made at intervals during the experiment, which lasted 30 days. These determinations were made

by the electric method described by the writer in Ecology, Vol. 7, No. 3, 1926. Briefly, it consists in impaling the insect upon a pair of fine electrodes set in a handle, as shown in Figure 10. When the electrodes penetrate the tissues a current of electricity is conducted to the poles and this causes a deflection within the galvanometer. It was shown by Zeleny (1909), when working on corn, and by the writer (1926) in his experiments on the moisture content of the tissues of living animals and growing plants, that the conductivity of the tissues is directly proportional to the amount of moisture present; so the magnitude of the deflection produced in the galvanometer when the electrodes

Fig. 10. Electrodes and Holder Used in Moisture Determinations

pierce the tissues indicates precisely the amount of moisture in these tissues. Therefore, by plotting a percentage scale of moisture for the species to be studied and placing this scale in the galvanometer, it is possible to read directly and quickly the percentage of moisture of the living tissues.

Tables IV and V show that the moisture content of both species of weevils was markedly affected by the moisture content of the wheat in which they live. These figures are averages obtained from 1000 separate moisture determinations. Every known precaution was observed in order to insure accuracy in making the determinations.

TABLE IV

EFFECT OF MOISTURE CONTENT OF WHEAT UPON MOISTURE CONTENT OF *S. oryza*

Per cent moisture of wheat	20.3	17.5	14.0	12.5	8.2	7.9	7.0	5.5	5.3	4.8
	Per cent moisture of weevils									
Start	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8
3rd day	57.3	57.3	57.9	59.4	62.2	62.5	67.2	68.8	70.9	*
4th day	...	...	58.1	64.6	69.4	69.4	68.8	68.5	67.9	
5th day	57.0	60.6	61.3	60.2	68.7	68.8	71.2	*	*	
8th day	57.2	58.9	62.1	61.8	70.4	67.7	71.0			
12th day	57.9	59.6	60.8	66.0	*	*	*			
21st day	58.7	58.9	60.3	66.0						
30th day	58.5	60.0	62.7	66.1						
	†	†	†	†						

* Dead.
† Alive.

The results obtained were the reverse of what was at first expected: the weevils living in the moist grain had the lowest moisture content, and a decreased amount of moisture in the wheat caused an outstanding increase in the moisture content of the weevils. Apparently, the reason for this is that grain containing less than a certain percentage of water is unfavorable for the weevils and consequently the metabolic processes

of the weevils are disturbed. This causes an undue breaking down of such tissues as fats, and brings about an excess of carbon dioxide and water as by-products. Since these insects normally use small amounts of water, their power of elimination is probably proportionately limited. Therefore this excess moisture can not be given off as rapidly as produced and it accumulates within the body. Marked increases in the moisture content were followed in every case by death, and decreases by continued activity. The latter conditions were presumably more favorable than even the original stock culture.

TABLE V

EFFECT OF MOISTURE CONTENT OF WHEAT UPON MOISTURE CONTENT OF *S. granarius*

Per cent moisture of wheat	20.3	17.5	14.0	12.5	8.2	7.9	7.0	5.5	4.8
	Per cent moisture of weevils								
Start	67.2	67.2	67.2	67.2	67.2	67.2	67.2	67.2	67.2
3rd day	58.5	58.5	58.0	58.5	62.8	64.4	67.6	69.3	70.5
5th day	58.9	59.5	61.5	61.3	65.1	67.2	67.6	71.8	71.4
8th day	58.7	59.5	60.6	61.4	66.0	66.0	67.6	72.5	*
12th day	58.9	60.6	61.6	62.5	66.3	66.9	71.4	76.9	
21st day	58.6	60.0	61.2	61.6	66.7	67.2	71.4	*	
30th day	59.1	60.2	61.5	63.0	67.9	71.4	*		
	†	†	†	†	†	*			

* Dead.
† Alive.

EFFECT OF MOISTURE CONTENT OF WHEAT UPON WEIGHT OF WEEVILS

In order to determine if the moisture content of the wheat would affect the weight of the grain weevils as it had affected their moisture content, the following experiments were conducted: Two samples each of wheat of 20.3, 14.0, 12.5, 8.2, and 7.0 per cent moisture were prepared, and in each were placed 50 weevils of either species which had just been weighed. At intervals of 2, 4, 6, and 19 days the weevils were taken out of the wheat and weighed, with results as shown in Tables VI and VII. Each time the weevils were removed from their containers a fresh supply of wheat of a similar moisture content was substituted.

TABLE VI

EFFECT OF MOISTURE CONTENT OF WHEAT UPON WEIGHT OF *S. oryza*

Per cent moisture of wheat	20.3	14.0	12.5	8.2	7.0
	Per cent weight of weevils				
Start	100	100	100	100	100
2nd day	102.5	101.2	97.3	93.1	87.9
4th day	105.0	101.2	94.7	84.9	79.2
6th day	105.0	101.2	89.4	73.9	63.4
19th day	105.0	103.8	*	*	*

* Dead.

With the higher percentages of moisture, namely, 20.3 and 14.0 per cent, the weight of both species, but especially of *oryza,* was not only maintained but actually increased. With the lower amounts of moisture, however, both species continued to lose weight until some individuals died when the experiments were terminated. The decrease in weight took place more rapidly with *oryza* than with *granarius.*

TABLE VII

EFFECT OF MOISTURE CONTENT OF WHEAT UPON WEIGHT OF *S. granarius*

Per cent moisture of wheat	20.3	14.0	12.5	8.2	7.0
	Per cent weight of weevils				
Start	100	100	100	100	100
2nd day	101.5	100.6	98.4	96.4	92.5
4th day	102.3	101.3	95.4	94.2	89.1
6th day	102.3	101.3	92.3	91.3	86.3
19th day	103.9	102.0	88.5	*	*

* Dead.

Davenport (1897) states "Water plays a part in growth second in importance to no other agent, so that in its absence growth cannot occur. As the quantity is increased, growth is increased until an optimum is reached. The amount imbibed does not, however, depend directly upon the amount available, but rather upon the needs and habits of the species."

For the decrease in weight an explanation is offered in the discussion of the previous experiment. The cause of the increase in weight can possibly be arrived at by reversing the order of reasoning. The fats which there were broken down are here built up. There, excess water was produced; here, the presence of additional water in the wheat provides the extra water necessary in building up the fats. Babcock (1912) states ". . . one hundred parts of cellulose or starch, $(C_6H_{10}O_5)_n$, containing 6.17 per cent of hydrogen, gives 55.5 parts of water; one hundred parts of anhydrous dextrose containing 6.66 per cent of hydrogen gives 60 parts of water, etc. Most of the fats yield more than their weight of water, while proteins, when completely oxidized, give from 60 to 65 per cent of water." Thus, on the one hand there is loss of weight through consumption of reserve tissues, and on the other hand gain in weight by the storing up of additional tissues.

FLUCTUATIONS IN MOISTURE CONTENT OF WHEAT AND RESPONSIVE CHANGES IN WEIGHT AND MOISTURE CONTENT OF GRAIN WEEVILS

It has been shown that a change in the moisture content of the wheat will cause a change in both moisture content and weight of the weevils. Further tests were made on the possibility of repeated fluctuations bringing about corresponding responses on the part of the weevils. Accordingly, 200 granary weevils were taken from the stock culture of 13.5 per cent moisture content and transferred first to wheat of 20 per cent moisture for 3 days, then put in 5.3 per cent wheat for 4 days; returned to 20 per cent wheat for 3 days, and once more put into 5.3 per cent wheat. The same procedure was used for the rice weevils except that the dried wheat contained 8.4 per cent moisture, 5.3 per cent being too low.

Several readings were taken each time before changing the wheat and the averages of these readings are plotted on charts in Figures 11 and 12. It can be seen that the moisture content of each species not only varied inversely with that of the wheat upon which they fed, as previously found, but that this continued as long as the changes were made.

For the study of the effect on weights, treatment similar to that described for moisture was adopted, and weights were taken at the time each transfer was made. In these experiments a direct variation in weight appeared, as in previous experiments, in response to fluctuations in moisture content of wheat; and these variations up and down continued as long as the changes were made. It is noteworthy that the largest amount of fluctuation in both weight and moisture content was always caused by the dry wheat, and that *oryza* was more affected than *granarius*.

The rapidity with which each species reacted to the varying conditions is another outstanding feature. Further experiments might show that these intervals could be still further shortened. Also the number of times that the weevils will react to varying moisture content of wheat might be extended, as each species was still active at the end of the series of experiments.

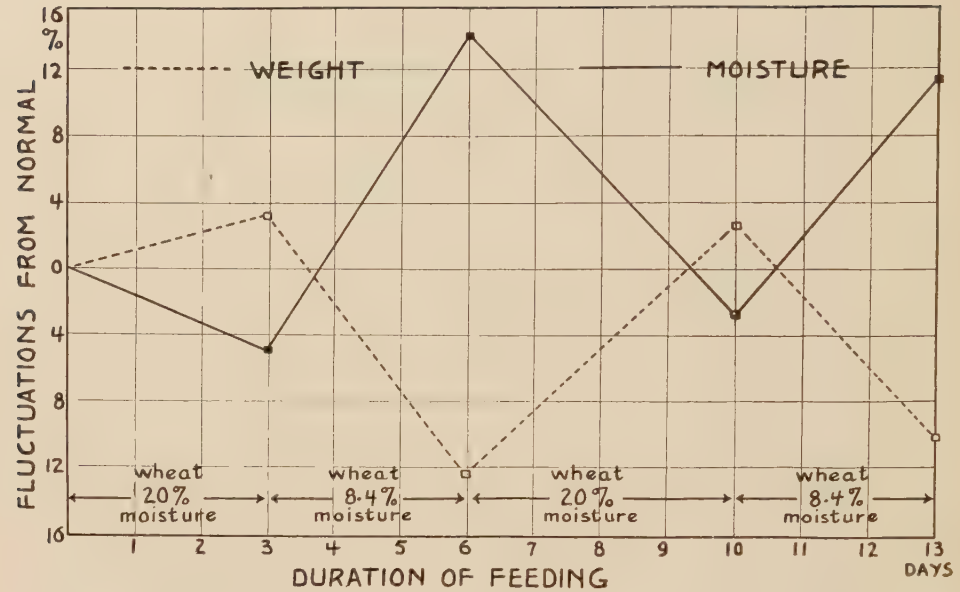

Fig. 11. Fluctuations in Weight and Moisture Content of *S. oryza* Following Changes in Moisture Content of Wheat

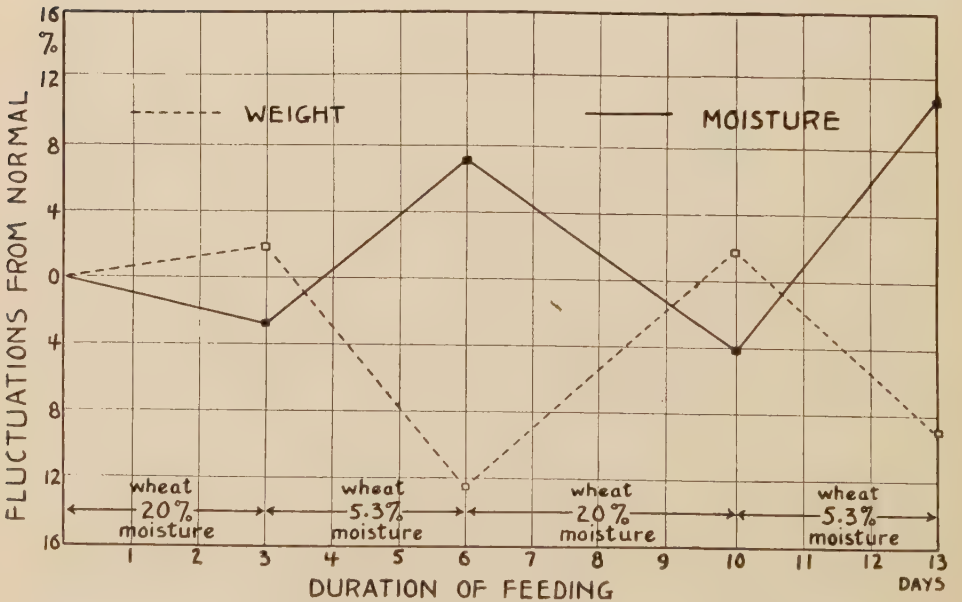

Fig. 12. Fluctuations in Weight and Moisture Content of *S. granarius* Following Changes in Moisture Content of Wheat

EFFECT OF MOISTURE CONTENT OF WHEAT ON FREEZING POINT OF GRAIN WEEVILS

In Tables IV and V is shown an inverse variation in the moisture content of the weevils with that of the wheat upon which they fed. This was exactly the opposite of what at first was expected, and required not only an explanation but, if possible, additional confirmation of the accuracy of the data.

An increase in the moisture content of the weevils would bring about a greater dilution of their body liquids; and a decrease in the amount of moisture would cause a greater concentration of their liquids. This would result in a corresponding change in the freezing point of their tissues: an increase in the moisture content would raise the freezing point and a decrease would lower it; so that, by determining the freezing point of a number of weevils taken from wheat of various moisture contents, the accuracy of the data showing an inverse variation in the moisture content of the grain weevils with that of the grain would be either confirmed or refuted.

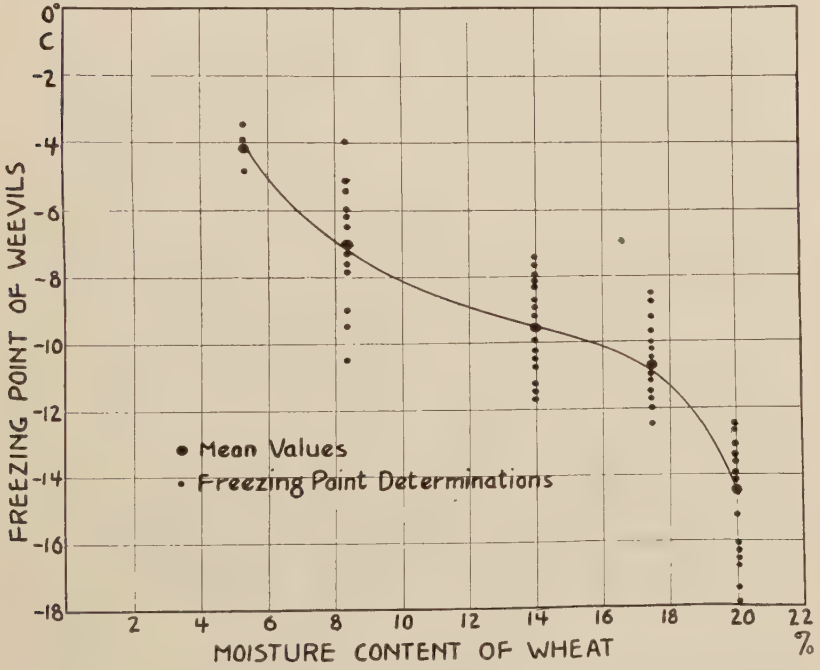

Fig. 13. Correlation of Moisture Content of Wheat and Freezing Point of Weevils

Accordingly, 75 freezing point determinations were made with insects taken from wheat of different moisture contents by means of an electric thermocouple. Results, as charted in Figure 13, show that

weevils living in dried wheat have a high freezing point and that by increasing the moisture content of the wheat the freezing point of the weevils living in it is lowered. These experiments, therefore, confirm the accuracy of the data in question.

In every instance the weevils failed to survive these freezing point determinations. Grain weevils, however, commonly endure for a short period temperatures as low as those at which freezing occurs. Therefore death apparently was caused by the lowering of the temperature below the freezing point to the undercooling point where the heat of crystallization is liberated, with the resulting rebound from the undercooling to the freezing point as described by Bachmetjew (1901).

It follows from these results that another disadvantage accrues to grain weevils in dry wheat. It is shown in the treatise that with dry wheat their mortality is increased and resistance to low temperature decreased, while here it is seen that their freezing point is raised. These conditions are detrimental to the weevils and increase their hazard of life.

OPTIMUM, MAXIMUM, AND MINIMUM MOISTURE CONTENT OF WHEAT REQUIRED BY GRAIN WEEVILS

Since the moisture content of the wheat has a marked effect upon the moisture content and weight of the weevils, the question arose as to what percentage of moisture would be most favorable; also how much and how little could be endured.

Samples of wheat with moisture content varying from 20.3 to 4.8 per cent, as shown in Tables VII and VIII, were prepared and 50 weevils of either species were placed in the containers—tin boxes 3 inches in diameter and 1½ inches high, with closely fitting lids. At the time of taking each count of the number of weevils living, the contents of the box were spread on a piece of white paper. The weevils usually feigned death at first but they were allowed 15 minutes to recover and move away. The active ones were then put back in the box with a fresh supply of wheat of the same moisture content.

TABLE VII

EFFECT OF MOISTURE CONTENT OF WHEAT UPON MORTALITY OF *S. oryza*

Per cent of moisture of wheat	20.3	17.5	14.0	12.5	8.2	7.9	7.0	5.5	5.3	4.8
	No. of living weevils									
Start	50	50	50	50	50	50	50	50	50	50
6th day	50	50	49	47	27	18	14	12	11	2
13th day	50	49	48	46	6	7		3		
23rd day	50	49	46	40	5					
38th day	50	49	46	39						

TABLE VIII

EFFECT OF MOISTURE CONTENT OF WHEAT UPON MORTALITY OF *S. granarius*

Per cent of moisture of wheat	20.3	17.5	14.0	12.5	8.2	7.9	7.0	5.5	5.3	4.8
	No. of living weevils									
Start	50	50	50	50	50	50	50	50	50	50
6th day	50	50	49	47	47	38	41	42	40	41
13th day	50	50	47	44	29	27	25	28	25	16
23rd day	50	49	47	44	27	20	16	18	16	4
38th day	50	49	47	44	13	4				

Results of the counts taken at intervals over 38 days show that neither species can endure a moisture content of below 8.2 per cent, and that about 14 per cent is probably necessary for *oryza*, while *granarius* can probably exist upon 12.5 per cent or slightly less. The comparatively high moisture content required by *oryza* and the lower amount which can be tolerated by *granarius* are possibly the explanation of the observation made by Dendy and Elkington (1920) that nearly all the adults of *S. oryza* kept indoors at ordinary laboratory temperatures were killed during the winter, while the adults of *S. granarius* survived the winter in large numbers. Wheat exposed in the laboratory here in Minnesota during the winter has been found to decrease in moisture from 14.0 to 8.0 per cent owing to the dryness of the atmosphere, which sometimes has a relative humidity as low as 15.0 per cent in winter. Dendy and Elkington attribute this mortality to temperature. However, unless long periods of temperature as low as 46° to 48° F. were included as being among the ordinary laboratory temperatures, it is improbable that the mortality was due to temperature, for the minimum effective temperature of *oryza* has been shown to be 46° to 48° F.

With regard to the optimum moisture requirements of grain weevils, if the criterion be that in which the least mortality occurred, it would be between 17.5 and 20 per cent. However, wheat having such a high percentage of moisture is inclined to heat, and it appears questionable whether the grain weevils, down through the centuries in which they have been known as pests of stored grain, have for their optimum a condition which is so seldom found in grain. It seems more likely that they have become adjusted through countless generations to a moisture content which most generally occurs and which is between 12 and 16 per cent.

In regard to the maximum amount of moisture which the weevils can endure in wheat, it is obvious that the study of the behavior of the weevils in grain of 25, 30, and 35 per cent moisture is mostly a matter of interest, for such high moisture content rarely, if ever, occurs

in stored wheat. Nevertheless, a few tests were made on very moist wheat and it was found that the maximum amount of moisture which the weevils can endure is not limited directly by the actual amount present, but by a complication which sets in under such conditions. When wheat was raised to 30 and 35 per cent moisture, the temperature increased from 72° to 79° F. in 4 hours, and the thousands of weevils which had been placed in the wheat all came to the surface and seemed much disturbed. This uneasiness could not have been due to the increased temperature, which was not at all excessive, but supposedly was due to the large quantity of CO_2 given off within the mass of wheat. Bailey and Gurjar (1918) prepared a chart showing that an increase in the moisture content of wheat above 15 per cent caused an enormous acceleration in the amount of CO_2 produced, owing to the increased rate of respiration of the wheat. The weevils remained chiefly upon the surface of the wheat for 4 days, during which time they went down into the mass for periods of about an hour but invariably returned to the surface and roamed around restlessly and died within a week.

ATTRACTIVENESS OF WHEAT HAVING VARIOUS PERCENTAGES OF MOISTURE

If samples of wheat varying between the two extremes of moisture were available to the weevils, would they select that which presented the most favorable conditions for them? To determine this, eight glass tubes 8 inches long and 1½ inches in diameter were marked on the outside into divisions numbered from 1 to 10. Into these divisions were placed samples of wheat as shown in Table IX.

TABLE IX

Moisture Content of Samples of Wheat Used in Tubes

Divisions	Moisture, %
1	4.8
2	5.3
3	5.5
4	7.0
5	7.9
6	8.2
7	12.5
8	14.0
9	17.5
10	20.3

When the divisions were half filled, 200 rice weevils were placed in each of four tubes and 200 granary weevils in each of the other four tubes. Then the remainder of each tube was filled. Thus the insects were centrally located and were at liberty to move in the direction to which they were attracted.

Corks were used in the ends of each tube, one having a 5/16-inch hole screened with wire gauze and the other being solid. The whole cork was placed in the moist end in half of the tubes and in the dry end in the other half. The tubes were then placed horizontally, away from the light, where they were left undisturbed, except for daily inspection, for 30 days.

The actual number of insects in each compartment of the tube could not be counted, as most of them were out of sight among the grain, but the light-colored excrement which always accumulates where these insects feed fell through the wheat to the lower side of the tube and thus revealed their presence. From the first day to the end of the experiment weevils lived only in sections 7 to 10, the greatest amount of excrement being found in No. 10 and grading down very noticeably to but little in No. 7. None ever fed in sections 1 to 6 and apparently they were repelled from that part of the tube, for no indication of their presence occurred at any time.

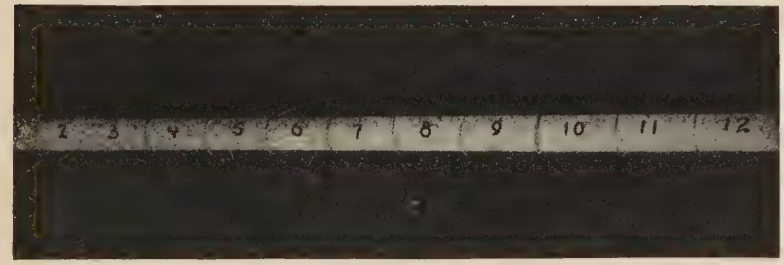

Fig. 14. Under Side of Tube Showing Graded Accumulation of Whitish Feces, Which Indicates Presence of Weevils

These experiments were repeated with a tube 24 inches long, with a view to increasing the distance apart of the two extremes and thus making it more difficult for the weevils to find their optimum. The divisions were increased to 12 by adding wheat of 25 and 30 per cent moisture, but in other respects it was treated the same as the previous tubes. The results were identical with those of the other experiments, a marked and early preference being shown for the moist wheat.

Figure 14 shows the condition of a tube at the end of three weeks. The weevils were left in the wheat for two months, during which time the moist wheat became moldy and then turned dark and began to decay, giving off a very offensive odor. Nevertheless, some weevils continued to live there. When the tube was emptied, the majority of the weevils were found in or near section 9, which contained wheat of 17.5 per cent moisture.

DRY WHEAT AND MORTALITY OF WEEVILS

Some data were needed on the cause of weevils being repelled from dry wheat and attracted to moist grain. The drier the wheat the harder becomes the seedcoat, and conversely, the more moist the grain the softer is its covering. Hard, dry grain is more difficult to penetrate in order to obtain food, and this mechanical difficulty probably aids in making dry wheat unfavorable and moist wheat attractive.

If the hardness of the grain covering, however, were the only factor involved, this could be discovered by using broken wheat. A series of experiments, therefore, was run in which dry grain of 8.2 and 4.8 per cent was used, and in half the experiments the wheat was left whole while in the other half it was well broken up. Check experiments having normal wheat of 13.2 per cent moisture and others in which no food at all was used, were run at the same time. One hundred weevils were placed in each container and the numbers were counted at intervals for twenty days.

The results are charted in Figures 15 and 16 and show that with *oryza* the death rate was practically identical in both whole and broken wheat of 4.8 per cent and all the weevils died within five days. This was one day earlier than where no food was provided, and the probable explanation is that the dry wheat lowered the relative humidity of the air in these containers and hastened death. In the whole wheat of 8.2 per cent moisture, death was retarded until the eleventh day, while in the broken wheat of similar moisture content 85 per cent of the

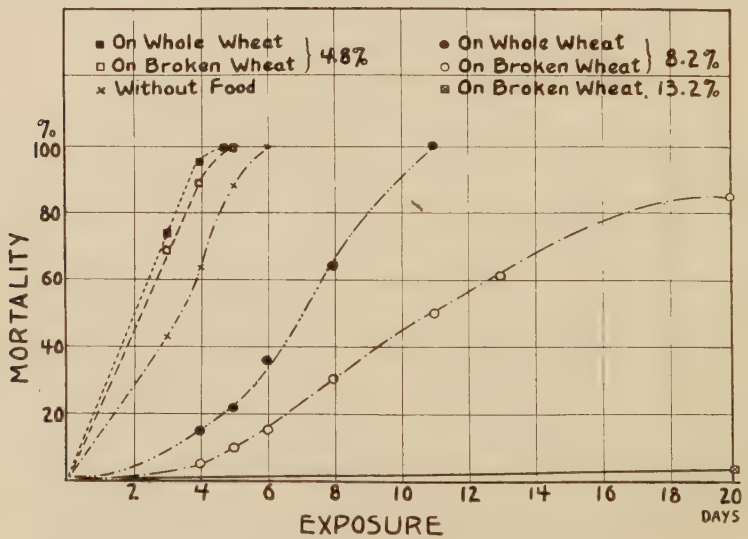

Fig. 15. Comparative Effect of Whole and Broken Dried Wheat on Mortality of *S. oryza*

weevils were killed in twenty days. The checks which lived in the 13.2 per cent wheat suffered only 2 per cent mortality. *Granarius* was able to endure the low moisture content of the experiments longer, and here, also, there is a slight advantage in favor of the broken wheat.

In all the experiments in which dried wheat was used, the rate of mortality increased each day whether the wheat was whole or broken, and it is evident that death was not due to the hardness of the pericarp and the consequent difficulty of obtaining food. It is more likely that death (preceded by loss of weight, as shown in Table VI and VII) was due to water deficiency. Jackson (1925) on pages 14-16, cites the work of several experimenters who found death to occur in various animals as a result of being fed on dry foods for certain periods. The importance of water in the metabolic processes has been emphasized by many workers. The living organisms require a large and fairly regular amount of water, and if this is lacking the body breaks down its reserve tissues to supply the water necessary. It was stated by Lorenzen (1887), as cited by Jackson (1925), that "a relatively dry diet is very effective in reducing the amount of fat in man, a principle used in the reducing diet by Oertel and others."

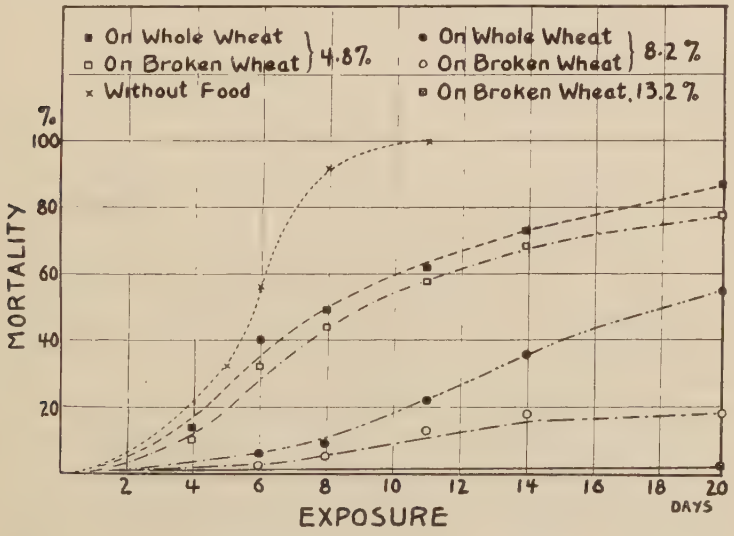

Fig. 16. Comparative Effect of Whole and Broken Dried Wheat upon Mortality of *S. granarius*

Pratje (1921) is quoted by Jackson (1925) as follows: "Pratje ('21) has recently made an extensive and careful study of the changes found in *Noctiluca miliaris* kept without food and observed alive. The normal fat-droplets disappear gradually (in eight to fourteen days), so that the cytoplasm becomes transparent. The central cytoplasmic mass

is greatly reduced in amount, with fewer and thinner radial extensions to the periphery. There is a corresponding increase in the peripheral vacuolar fluid, however, so there is no appreciable decrease in the size of the whole cell. The nucleus becomes more distinctly visible and more transparent. The nucleoli often become visible. Thus far only the more fluid endoplasm has been affected, but as inanition progresses, the firmer, peripheral protoplasmic structures (cell membrane and organs) are attacked and consumed. In about three weeks, all available sources of energy are exhausted. Recovery by refeeding is now impossible, and death soon occurs."

Berger (1907) fed the larvae of the mealworm, *Tenebrio molitor,* on dried bran for a time and found that their weight decreased each day until death occurred. He also found the moisture content of these insects to remain nearly constant, altho they lost weight until death occurred.

Bodine (1921), when working with certain species of grasshoppers, found that death from starvation could be deferred for about twice the length of time if water were supplied.

It therefore seems probable that in these experiments death was due to the breaking down of such tissues as fats to supply the required amount of moisture. This, the results of the previous experiments tend to confirm.

EFFECT OF EXPOSURE TO LOW TEMPERATURE UPON MOISTURE CONTENT OF GRAIN WEEVILS

In the daily study of the moisture content of the weevils during the course of exposure to low temperatures, a marked disturbance of their metabolic processes is revealed by the fact that during the first few days of dormancy a very rapid rise in moisture content takes place, followed by an equally rapid decline, as shown in Figure 17. In addition, a check series was run in which both species were allowed to starve at a room temperature of 72° F. (Fig. 18). With *oryza* death occurred in every instance while they had an abnormally high percentage of moisture, which proves that death from low temperatures is not caused by loss of moisture. *Granarius* were still alive after their moisture content had returned to normal; and the long, gradual decrease of moisture which preceded death does not indicate that death was caused by loss of water, for even at the time of death the amount of moisture present was identical with the amount commonly found in weevils when living under the most favorable conditions.

The fluctuations in moisture content during exposure to low temperatures may be considered along with two other occurrences, namely, the continuous loss of weight which has been shown to occur, and the death of the insects, which is inevitable if dormancy continues. It

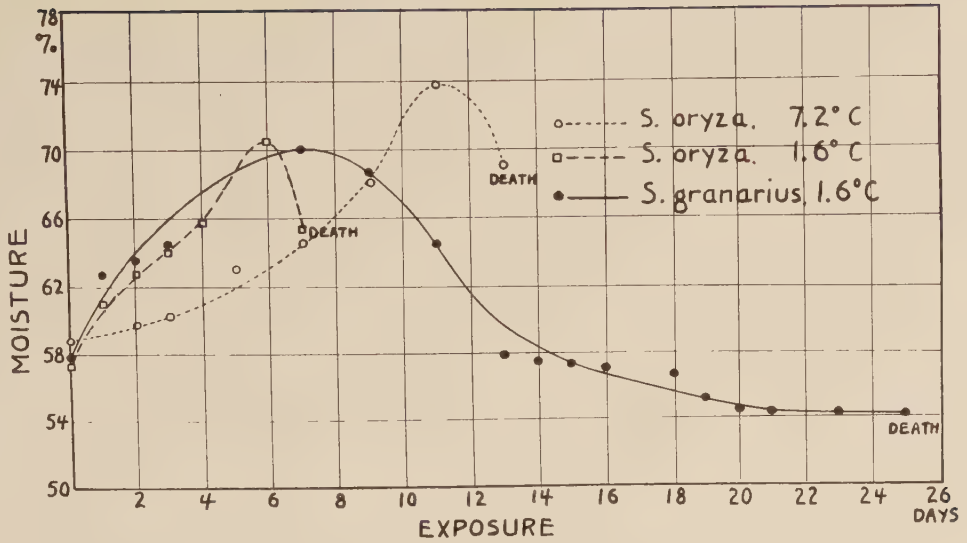

Fig. 17. Disturbing Effect of Moderately Low Temperatures upon Moisture Content of Dormant Weevils

appeared earlier that these insects have never learned to adapt themselves to low temperatures and that their metabolic processes are probably not reduced in velocity to the very low rate characteristic of hibernating insects when in the state of dormancy. Thus, there are on the one hand the active metabolic processes which require a supply of oxidizable organic material, and on the other hand the inability of the insects, being dormant, to feed themselves. Metabolism therefore is maintained at the expense of such reserve tissues as fats, stored in the body. This accounts for the continuous loss of weight. Carbon dioxide and large amounts of water are formed as by-products; and

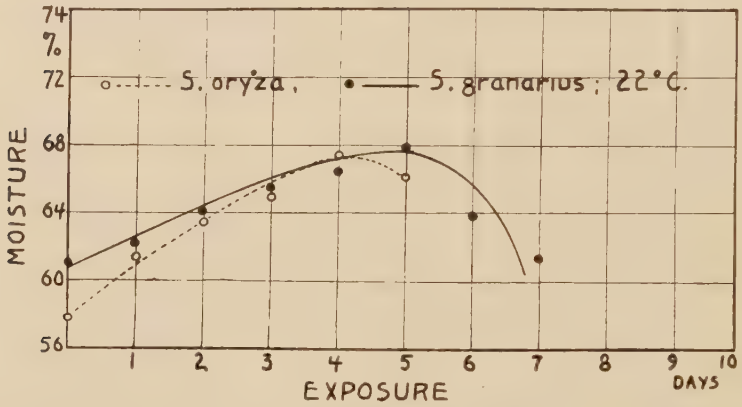

Fig. 18. Rise in Moisture Content of Active Weevils During Starvation and Decline with Approaching Death

since these insects live in grain which seldom contains more than from 12 to 16 per cent moisture, their capacity to eliminate water is probably very limited, and the excessive amount of water which results from the oxidation of fats accumulates within the body and causes the high moisture readings which are so outstanding in this work.

EFFECT OF MOISTURE CONTENT OF WHEAT ON RESISTANCE OF GRAIN WEEVILS TO LOW TEMPERATURE

Some data have thus far been accumulated regarding the effect upon moisture content and weight of grain weevils of two factors, viz., the moisture content of their food and exposure to low temperatures; and some hypotheses have been offered to account for what has happened. Meager as the facts are likely to be, they appear to fit together and each helps to make more intelligible some of the phenomena associated with it. In addition, it has been possible, with the data already acquired, to prove by experimentation that the moisture content of the wheat affects the ability of the weevils to resist exposure to low temperatures.

A large number of weevils of each species were placed in wheat of different moisture content and allowed to feed there for about three weeks. They were then removed, along with a check series from wheat of normal moisture, to the refrigerating cabinet and exposed to a given low temperature. In every instance the moisture content of the wheat had a definite effect upon the cold-resistance of the weevils.

Figure 19 shows the results of 58 experiments with 9931 granary weevils. The resistance of those which had lived in wheat of 17.2 per cent moisture was so greatly increased that, for instance, an exposure of from 20 to 40 hours to —12.2° C. (20° F.) caused only 10 per cent mortality, while those which had fed upon wheat of 13.1 per cent moisture suffered a mortality as high as 80 per cent.

With the rice weevils a more extended series was conducted in which the temperature was —1.1° C. (30° F.).

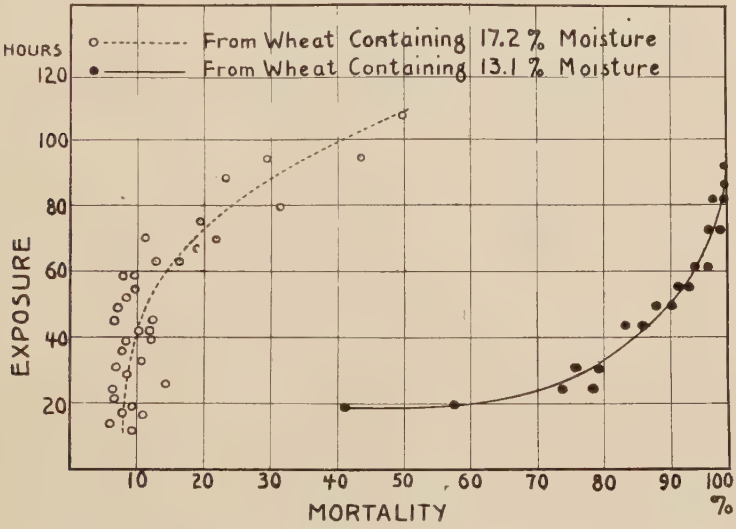

Fig. 19. Comparative Resistance of *S. granarius* to —12.2° C. (10° F.) When Fed on Moist and Normal Wheat

Figure 20 shows that with a decrease or increase in the moisture content there is a corresponding decrease or increase in the resistance of the weevils to low temperatures.

An additional fact came out of this experiment, namely, the correlation of the moisture content of wheat and the time required to obtain 100 per cent mortality takes the form of practically a straight line, as in Figure 21. From this it appears possible, when knowing the moisture content of the wheat, to predict the time required to kill; and conversely, having established the length of exposure necessary to kill, the percentage of moisture of the wheat could be determined.

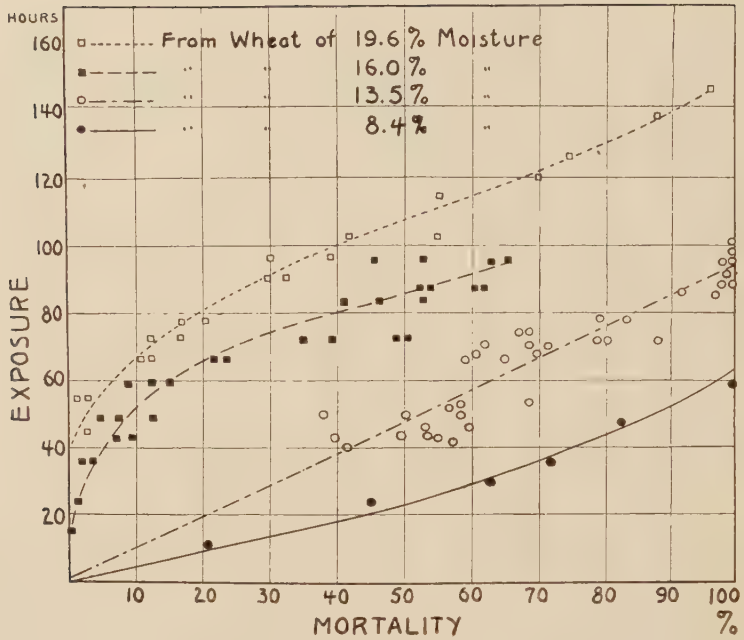

Fig. 20. Comparative Resistance of *S. oryza* to —1.1° C. (30° F.) when Fed on Wheat of Various Moisture Contents

Moisture of wheat, per cent	No. of experiments	No. of individuals
19.6	20	6,007
16.0	32	8,941
13.5	51	11,362
8.4	6	1,194
	109	27,504

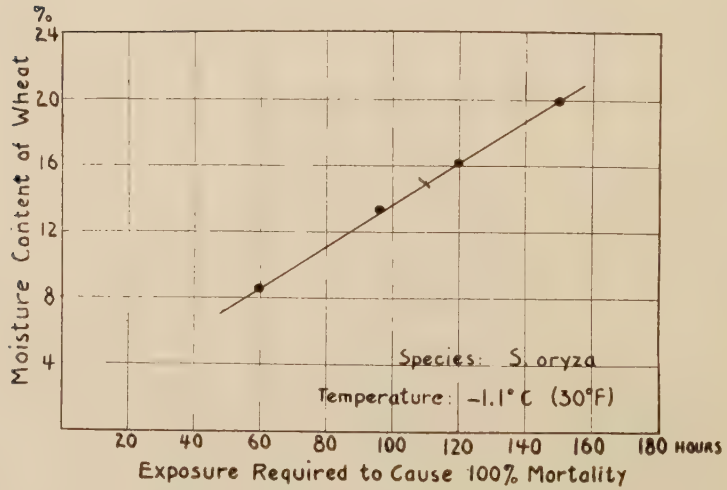

Fig. 21. Correlation of Moisture Content of Wheat and Length of Exposure Required to Kill Grain Weevils

COMPARATIVE MOISTURE-RETAINING CAPACITY OF GRAIN WEEVILS

It is characteristic of *oryza* to be much more susceptible than *granarius* to fluctuations in the moisture content of its food. This susceptiblity is accompanied by a high mortality if the moisture content is reduced to less than its normal requirements. This might be due to the greater loss of moisture which occurs with this species, partly due no doubt to the evaporation of a greater amount of moisture from its surface. A thinner or a more porous integument, or the presence of larger spiracles would facilitate the exudation of moisture; and being a smaller species, *oryza* has a greater amount of surface to mass. A morphological study of the structure of these insects is beyond the scope of the present study, and other means were adopted with a view to determining the comparative rate of moisture loss.

This was done by studying the rate at which the moisture decreased during desiccation. A large number of weevils of each species were obtained and moisture determinations taken; then they were chloroformed so that death would be simultaneous. Subsequent determinations were made until the weevils became dry.

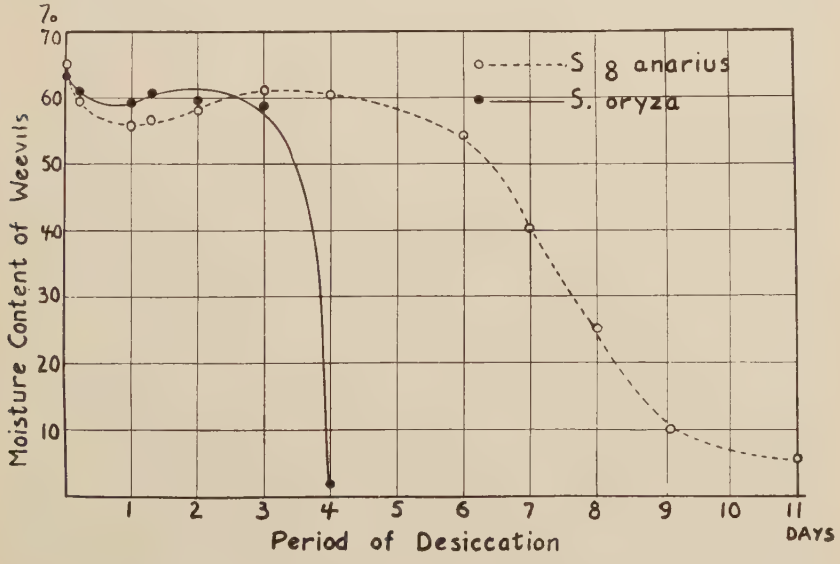

Fig. 22. Comparative Rate of Drying Out of Tissues after Death

As usual, *oryza* lost its moisture rapidly, and became entirely dry in 4 days; while *granarius* retained moisture for 11 days. A study of Figure 22 will show this relation. The very rapid descent in moisture during the first three hours is doubtless due to the desiccating effect of the chloroform upon the tissues. With the evaporation of the chloro-

form, the moisture content rose again somewhat, but not to the original amount. The absence of the rise in moisture which is shown in Figures 17 and 18 is noteworthy and may be attributed to the cessation of the metabolic processes after death.

EFFECT OF WEEVIL INFESTATION UPON MOISTURE CONTENT OF WHEAT

Having found that the moisture content of the wheat materially affects the moisture content of the weevils' tissues, it was then to be determined if the reverse of this occurs. The present experiment was planned to discover if the moisture content of the grain was affected by the presence of weevils living and feeding within its mass.

Thirteen bottles were each filled with 1000 grams of wheat having a moisture content of 13.5 per cent. In each of 12 bottles, 1000 weevils were placed (6 bottles for each of the two species) and the 13th was left uninfested to serve as a check. These bottles were allowed to stand undisturbed for two months, when the weevils were removed and determinations were made of the moisture content of the wheat. These determinations showed that the granary weevil had raised the moisture content to 17.3 per cent and the rice weevil to 18.0 per cent, while the wheat in the check or uninfested bottle decreased to 13.1 per cent, owing to drying out in the laboratory.

This shows that the grain weevils are able to initiate a rise in the moisture content of the wheat, despite the dry atmosphere of the laboratory. The assumption is that this was done through the elimination of moisture produced during the process of respiration, not necessarily the respiration of the weevils only, but also of the wheat. Back and Cotton (1924) state that the temperature of grain is frequently raised when a large number of weevils collect together in the mass of grain, and that this must be due to the activities of the insects themselves for it can be lowered by fumigation. Phillips and Demuth (1914) observed that clusters of bees will raise the temperature to 95° F. within the hive when the outside temperature is 32° F.

With this increase in temperature of the grain by the presence of weevils there occurs an acceleration of respiration of the wheat as shown by Bailey and Gurjar (1918), and this in turn gives off carbon dioxide and water. Therefore it seems probable that part of the increase in moisture content of the wheat results directly from the respiration of the wheat itself.

The fact of the increase of moisture content of wheat by the presence of weevils is of more than passing interest for it has an important corollary, viz., it brings about a series of conditions which are all more favorable for the weevils themselves. These are:

(1) The moisture content of the wheat is raised to one which these experiments show to be more favorable in prolonging the life of the weevils.

(2) The weight of the weevils is increased, which implies a greater amount of reserve tissue such as fats being formed and a correspondingly greater protection from starvation.

(3) Resistance to low temperatures is greatly increased. In this particular case it was increased over 200 per cent, for the weevils used in the experiment charted in Figure 19 were obtained from the identical culture that was raised from 13.5 to 17.7 per cent through metabolic water.

(4) The freezing point of the insect tissue is lowered.

(5) Sexual activity is stimulated in the higher moisture contents.

(6) The temperature is raised following an increased moisture content to one more favorable for development. Bailey and Gurjar (1918) show that biological oxidation of the wheat through the action of oxidizing enzymes is accelerated by increased moisture, and this raises the temperature of the grain.

The temperature of a culture of rice weevils in a north room away from direct rays of the sun was taken for 12 days during the month of September, and when compared with the temperature of the room air close by the tank showed an increase in every case, as follows:

TABLE X

Comparison of Temperatures of Laboratory and Culture of Rice Weevils

Date	Laboratory	Culture
	° C.	° C.
September 16	22.5	31.0
17	27.0	34.5
19	24.3	32.0
21	17.0	27.5
23	19.2	27.0
24	21.5	31.2
28	20.5	30.2
Average	21.7	30.2

It therefore appears that if these insects can once become established they will not only maintain themselves but will continue to improve their condition. An obvious disadvantage lies in the fact that with a limited food supply the saturation point, followed by death, is reached more quickly.

TEMPERATURES OF GRAIN STORED IN TERMINAL ELEVATORS, WITH THE SUGGESTION OF A POSSIBLE MEANS OF CONTROLLING GRAIN WEEVILS

Experiments in the laboratory show that grain weevils are killed by exposure to temperature as high as 45° F. for *oryza* and 35° F. for *granarius*. It follows from this that if the temperature of grain in storage is reduced to that degree and held there long enough, the weevils infesting the grain will be destroyed.

A preliminary study of the temperatures of grain in elevators was made from records supplied by one of the elevator companies of Minneapolis, whose tanks are equipped with a thermocouple system which registers the temperature of the mass of grain every five feet up the tank. Records of the temperature were taken weekly throughout the year.

These temperatures were plotted for the year and one of the charts is shown in Figure 23. This chart can be divided into three sections. In the first part the temperatures are increasing in the upper part of the tank and in this section occurs the greatest spread between the highest and lowest temperatures, the lowest being 37° F. and the highest 85° F. Such a high temperature as the latter indicates that the wheat is getting into a dangerous condition, one remedy for which is overturning, so about November 24, when the outdoor mean temperature was 35° F., the wheat was turned from tank 7 to tank 12. This reduced the temperatures to 40° F. for the lowest and 65° F. for the highest, and diminished the range to 25° instead of 48° F. as before the overturning. It is thus seen that the overturning caused a general decrease in temperature of the wheat throughout the tank.

In the third section is seen the effect of an additional overturning when advantage was taken of a drop in the outside temperature to lower the temperature of the wheat still further in order to carry it over the coming summer. The lowest and the highest temperatures are still further reduced to 35° and 54° F. respectively, with a range of 21° F. In the upper 20 feet of the tank the temperatures are low enough to kill both species of weevils. It is very probable that if an additional overturning or two had been made during January, when the outside temperatures were down to 0° F., the temperatures of the grain within the tank would all have been lowered to about 35° F., and in such a temperature no weevils could remain alive for more than a month. This wheat, being free of infestation, could be gradually raised in temperature by additional overturnings in early spring to avoid the "sweating" which occurs when cold wheat is exposed to the air in summer.

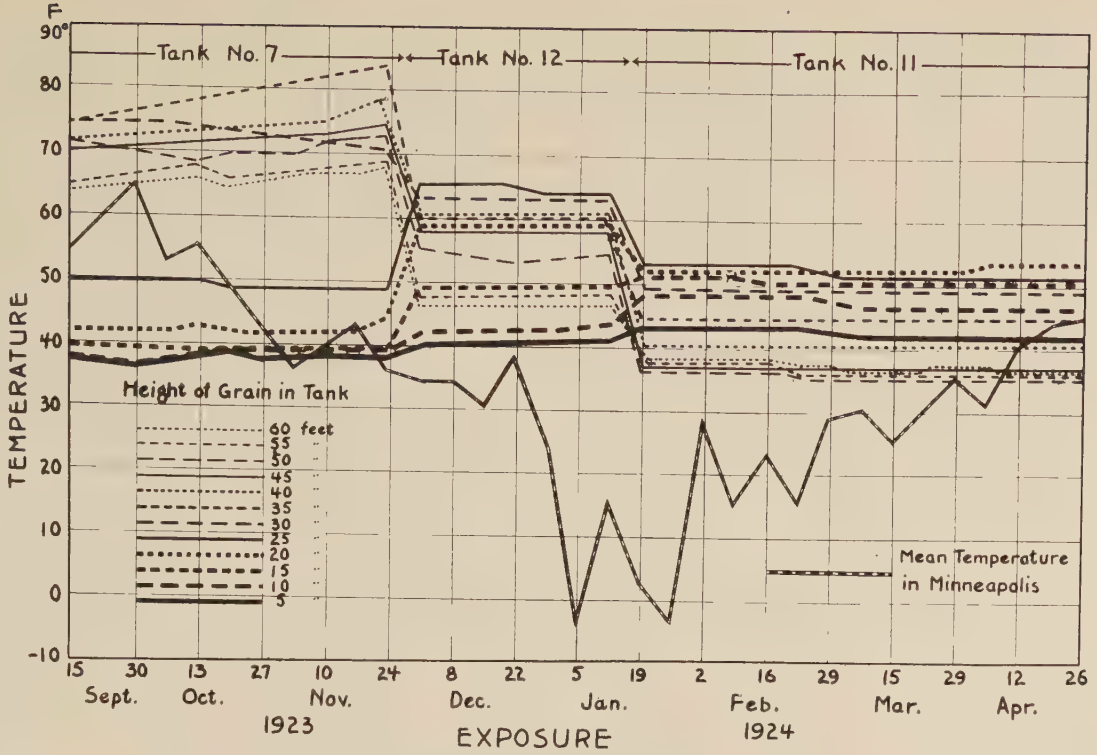

Fig. 23. Temperature of a Sixty-Foot Tank of Wheat During a Period of Seven Months

SUMMARY

A description is given of the methods and the equipment used in the experiments; and the various aspects of the subject are outlined.

In the series of abrupt exposures, where the temperatures were dropped suddenly, there was a well marked correlation between length of exposure and percentage of mortality. This is true for every temperature used.

Both species are unable to endure dormancy. *Oryza* becomes dormant at approximately 7.2° C. (45° F.) and *granarius* at 1.6° C. (35° F.); and in this condition they will perish in seventeen and thirty-eight days respectively.

During exposure to low temperatures, a continuous loss of weight occurs with each species, and this loss is regular and constant.

The gradual approach of cold weather, which "hardens" hibernating insects and enables them to survive the very low temperatures of winter, has the opposite effect upon grain weevils (and probably the whole group of non-hibernating insects) and causes death to occur more rapidly.

If the period of dormancy be broken and normal conditions be returned before death occurs, the accumulated injury can be overcome. In addition, the original vigor can be increased if the new environment be a more favorable one.

The moisture problem of grain weevils is considered.

The moisture content of grain has a marked effect upon the moisture content of the weevils. Those living in moist grain had the lowest moisture content, and a decrease of moisture in the wheat caused an outstanding increase in that of the weevils.

Weevils living in moist grain gain in weight while those in dry wheat continually lose weight.

Fluctuations in the moisture content of the wheat cause responsive changes in the weight and moisture content of the tissues of the grain weevils. Weight varies directly and moisture content varies inversely as the moisture content of the wheat. Responses continue as long as changes are made in the moisture content of the wheat.

The freezing points of grain weevils are affected by the moisture content of the grain upon which the weevils feed. Dried wheat raises and moist wheat lowers their freezing points.

Optimum, maximum, and minimum moisture content of wheat required by grain weevils are investigated. *Oryza* is more sensitive to dryness in wheat than *granarius* but neither can endure a moisture content as low as 8.2 per cent. Probably 14.0 per cent is necessary for *oryza,* while *granarius* can exist upon 12.5 per cent or slightly less. The optimum has not been definitely ascertained but is possibly around

17.5 per cent. The maximum amount is probably not more than 25 per cent, for above that amount a complication sets in by the production of carbon dioxide through increased respiration which is injurious to the weevils.

In the experiments on the attractiveness to weevils of wheat of various moisture content, it is found that moist wheat attracts and dry wheat repels.

Dry wheat causes death of the weevils, not because of the hardness of the grain covering and the difficulty of obtaining food, but through lack of moisture which brings about a breaking down of the fatty tissues of the weevils to supply the moisture required.

The moisture content of the wheat has been found to affect the ability of the weevils to resist low temperatures. Moist grain increases and dry grain decreases resistance. Correlation of moisture content of wheat and time required to cause 100 per cent mortality takes the form of a straight line.

Moisture-retaining capacity is tested by desiccation of tissues immediately after death. With cessation of life processes any individual variations are probably eliminated and a more reliable test can be made of the properties of the body structure itself to resist desiccation. *Oryza* loses its moisture content in four days while *granarius* retains some moisture for eleven days.

Weevils raise the moisture content of the wheat upon which they feed, and this results in the formation of a chain of interesting conditions each of which reacts upon the weevils themselves.

A chart is shown on which are plotted temperatures of grain in a terminal elevator in Minneapolis. This covers a period of seven months and shows that by running the grain from one tank to another during cold weather the temperature of the grain is much reduced. A suggestion is made that reducing the temperature of grain to below the minimum for the weevils might be an effective means of control.

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BIOGRAPHY

William Robinson was born in Hamilton, Ontario, Canada, on Sept. 7, 1896. He lived for the most part in Toronto and Guelph where he received his public and high school education. Later he took a business college course and spent the next four years in business. Following this he attended the Agricultural College of the University of Toronto and was graduated with the degree of B.S.A. in 1918. He then received an appointment in the Dominion Civil Service with the Entomological Branch at Vineland, Ontario, and worked there for the next five years on deciduous fruit insects. In 1923 he went to the University of Kansas, where he received the degree of M.S., and in 1924 to the University of Minnesota where he obtained the degree of Ph.D.

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